REMARKS/ARGUMENTS

Claims 1-7, 16, 18 and 20-21 are pending. The claims have been amended. No new matter has been introduced. Reexamination and reconsideration of the present application is respectfully requested.

Claim Rejections

Claims 1-7, 16, 18 and 20-21 are pending. Claims 1-7, 16, 18 and 20-21 have been rejected. Claims 1, 3, 6-7, 16, 18 and 20 have been amended.

The Examiner noted that the claims contain some errors, such as in claims 1 and 18. The Applicant has corrected these errors and thanks the Examiner for notifying the Applicant.

35 U.S.C. §112, first paragraph

The Examiner rejected to the claims 1-7, 16, 18 and 20-21 as not being enabling for alleviating or preventing ammonia accumulation in any and all environment. The Applicant has amended the claims to recite "an aquarium." There is support throughout the application for using the disclosed strains to prevent or alleviate ammonia accumulation in aquariums. Furthermore, the Examples teaches how the inventors experimented as such with the bacterial strains (Examples 1, 9 and 11-16).

Moreover, the Applicant has amended the claims to specify the conditions under which the rDNA hybridizes. Support for the amendments can be found on pages 16 (lines 21-28), 27 (lines 4-12; Table 10), and 28 (lines 1-13) of the specification, where probe and hybridization conditions are disclosed. What is disclosed in the specification is well within the capabilities of one of skill in the art to make and use the invention as claimed. The specification discloses how the probes are developed and the condition parameters under which hybridization occurs. Page 27, Example 6. For example, the specification states "[i]n situ hybridization of the fixed, immobilized cells was carried out in a hybridization solution consisting of 0.9 M NaCl, 20 mM Tris/HCl (pH 7.4), 0.01% sodium dodecyl sulphate (SDS), 25 ng of oligonucleotide probe, and varying amounts of formamide...To achieve the same stringency during the washing step, as in the hybridization step, the wash solution contained 20mM Tris/HCl (pH 7.4), 0.01% SDS, 5 mM

EDTA, and NaCl. The concentration of NaCl varied according to the percent formamide used in the solution. For 20% formamide the NaCl concentration was 215 mM, for 30% it was 120 mM, and for 40% the NaCl concentration was 46 mM...The optimum stringency was determined to be 30% formamide for the S-G-Nsspa-0149-a-A-18 probe. For the S-G-Nsspa-0149-a-A-19 probe the optimum stringency was determined to be 20% formamide. The optimum stringency was determined to be 20% formamide for the probe represented by SEQ ID NO:21, and 20% formamide for the probe represented by SEQ ID NO:24." Page 29, lines 2-23.

By using the specified hybridization conditions together with well-known techniques, the one of skill in the art would be able to identify the strains sharing the specified homology. These methods and techniques are routinely practiced in the art (*e.g.*, alignment search tool (BLAST) (S.F. Altschul et al. 1990. Basic local alignment tool. J. Mol. Biol. 215:403-410) and CHECK_PROBE (B.L. Maidak et al. 1994. The ribosomal database project. Nucleic Acids Res. 22:3485-3487.)). By using tools and protocols commonly known in the art, one skilled in the art can then readily test and see whether a bacterial strain sharing the claimed homology and hybridizing under the specified conditions exhibits ammonia-oxidation. Moreover, the Examples teaches how the inventors detected and measured the presence of ammonia-oxidation capabilities in bacterial samples (Examples 11-16).

In addition, one of skill in the art understands that the 16S rDNA is a highly conserved region of gene, and has been used by those skilled in the art to discern and describe phylogenetic relationships, rather than functional comparisons, which impart information regarding which regions of the 16S rDNA are variable at the species level (Woese *et al.*, Proc. Nat'l. Academy Sci. 74(11): 5088-5090 (1977)). As such, it is known to one of skill in the art which regions of the 16S rDNA gene are universally conserved between species. The specification teaches how this concept is used to construct probes that target a specific homology to the 16S rDNA which determines the phylogenetic relationship of the bacteria with that sequence. Pages 25-27.

The use of 16S rDNA to determine phylogenetic relationships of species is well-known in the art (Teske *et al.*, J. Bact. 176(21): 6623-6630 (1994)). In Teske *et al.*, the 16S rDNA sequences of ammonia- and nitrite-oxidizing bacteria are compared in order to show phylogenetic relationships. By sequencing that gene of known AOB and NOB, it was shown that almost every known AOB fell into the beta-subdivision of the *Proteobacteria* but that these bacteria could be distinguished from other beta-subdivision non-AOB based on the 16S rDNA

sequences. This is important to show that beta-subdivision AOB form a group within the beta-*Proteobacteria* that includes no other non-AOB, while different species of AOB can be distinguished based on their 16S rDNA sequence.

The data presented in the present specification shows that SEQ ID NO:1 and its variants is discovered to fit into the larger beta-subdivision AOB group and thus one of skill in the art would recognize an organism identified by SEQ ID NO:1 and its variants as an ammonia-oxidizing bacteria. The Examples show that the isolated and purified organism does indeed oxidize ammonia and thus confirms this designation. The comparison of the known 16S rDNA sequences in Teske *et al.*, by aligning the sequences, demonstrate that only very specific regions of the bases vary. As mentioned above, by using the framework developed by Teske *et al.* and known tools such as BLAST, it was discovered that SEQ ID NO:1 is similar in the regions shared among known AOB but varies in those specific regions that distinguish AOB species (4% or less). This phylogenetic comparison is shown in Figure 1 of the specification. Thus, given what is taught about SEQ ID NO:1 in the specification, the specified hybridization conditions that are required, and the examples demonstrating how ammonia-oxidation level is analyzed, one skilled in the art would be able to readily synthesize and identify the variants of the reference sequence which share the claimed homology and also exhibit ammonia-oxidation.

Salt requirement

The Examiner states that SEQ ID NO:1 and its variants appear to be salt-requiring. This is inaccurate as SEQ ID NO:1 and its variants are freshwater bacterial strains. The specification discloses that the strains were isolated from freshwater environments. For example, the specification discloses in the Examples that SEQ ID NO:1 was collected from a R7 biomass, which was seeded originally from a freshwater biofarm (page 34, Example 9; Table 12). Thus, the strains are not salt-requiring.

In light of the above considerations, Applicants respectfully submit that the present claims meet the enablement requirement of patentability. It is therefore respectfully requested that the Examiner's rejection of claims 1-7, 16, 18 and 20-21 based upon the enablement requirement of patentability be withdrawn.

Conclusion

This response is being submitted within the three month deadline. In the case any fee is owed, please charge deposit account number 03-3975 (ref. 81289-294309). The Applicant

believes that claims 1-7, 16, 18 and 20-21 are now in condition for allowance, and a favorable action is respectfully requested. If, for any reason, the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (213) 488-7100 to discuss the steps necessary for placing the application in condition for allowance should the Examiner believe that such a telephone conference would advance prosecution of the application.

Respectfully submitted,

PILLSBURY WINTHROP SHAW PITTMAN LLP

Date: 11/20/2006

By: _____
Carolyn S. Lu
Registration No. 56,817
Attorney For Applicant(s)

725 South Figueroa Street, Suite 2800
Los Angeles, CA 90017-5406
Telephone: (213) 488-7100
Facsimile: (213) 629-1033

Enclosures: Woese *et al.*, Proc. Nat'l. Academy Sci. 74(11): 5088-5090 (1977); Teske *et al.*, J. Bact. 176(21): 6623-6630 (1994).